

Atty. Docket No.: 8039/1070

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Tomlinson, et al.
Serial No.: 09/511,939
Filed: February 24, 2000
Entitled: Method to Screen Phage Display
Libraries with Different Ligands

Examiner: Ponnaluri, P.

Group Art Unit: 1639

Conf. No.: 5170

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF IAN M. TOMLINSON UNDER 37 C.F.R. §1.131

I declare:

1. I, Ian M. Tomlinson, am an inventor of the invention claimed in the above-noted U.S. Patent application.
2. I have read and understood the Office Action mailed July 11, 2003 and have read and understood the cited reference, U.S. Patent No. 6,057,098 (the "'098 patent;" issued to Buechler et al. on May 2, 2000 from an application filed April 4, 1997). I understand that the Examiner has cited the '098 patent as a novelty reference over claims 33-41 and 44-52.

The '098 patent is cited as teaching methods of producing a multivalent polypeptide display library comprising a library of phage representing tagged fusion proteins. The Examiner states that the tag can be any polypeptide with a known receptor showing high binding specificity for the tag (referring to column 7, lines 10-11). The Examiner further asserts that Buechler et al. teaches contacting the library with a receptor (which the Examiner characterizes as analogous to a generic ligand) and separating bound members of the library from unbound members to produce a sublibrary of polypeptides. The Examiner states that the selected sublibrary is then screened by contacting the library with a target and separating the library members bound to the target via their displayed polypeptides.

3. Prior to the April 4, 1997 filing date of the '098 patent, I had conceived of the invention as claimed in claims 33-41 and 44-52. The invention was reduced to practice with diligence shortly thereafter. The attached Exhibit 1 consists of copies of my notebook entries detailing the experiments that gave rise to the claimed invention. The dates on this exhibit have been redacted.

4. As documented in paragraphs A-N below, and in the accompanying exhibits, the object of the invention is to improve polypeptide library technology to increase the likelihood of identifying polypeptide molecules that bind desired targets. Many polypeptide libraries are made by introducing diversity into the binding site of a known polypeptide structure and typically this is performed using degenerate oligonucleotides and sometimes also the polymerase chain reaction. One of the problems facing those working with such libraries is that a large proportion of the polypeptides in the library are in fact non-functional and are unable to bind to a target ligand. Non-functional molecules can arise due to the introduction of amino acids into the binding site (encoded by the degenerate oligonucleotides) or in the framework regions (by errors caused by the PCR amplification process or in the oligonucleotides used to amplify the polypeptide-encoding genes) that prevent or perturb the ability of that molecule to fold properly. Alternatively, non-functional polypeptide molecules can be created by the introduction of frame-shifts or stop codons that prevent or perturb the expression of the polypeptide. The ratio of non-functional to functional members in a given library can result in a high background of non-functional polypeptide molecules during the isolation of functional polypeptides against a given target ligand. Thus, a method that increases the proportion of functional and properly folded molecules in a library will increase the likelihood of identifying polypeptide molecules that bind a given target ligand. One area of polypeptide library technology that would benefit from a method that increases the proportion of functional and properly folded polypeptides is antibody library technology, because antibodies typically involve both heavy and light chain domains that each must be diversified and that each must properly fold in order to generate a functional binding molecule.

Prior to April 4, 1997, I conceived an approach to overcome this problem. In this approach, a sub-library of antibody Heavy chains is selected for members that properly fold, a sub library of Light chains is selected for members that properly fold, and the two selected sub-

libraries are combined to form a library of antibodies that have been pre-selected for folded members that is then selected for binding to target antigen. The pre-selection for proper folding is performed by binding the members of each sub-library to a generic ligand that only binds properly folded sub-library molecules. For example, the Heavy chain sub-library can be pre-selected with Protein A, and the Light chain sub-library can be pre-selected with Protein L. In one approach, scFV constructs are employed.

In order to select sub-libraries that properly fold, two scFv vector constructs are required:

- a) a construct encoding a known "dummy" Heavy chain can be used to generate the sub-library of diverse Light chains; and
- b) a construct encoding a known "dummy" Light chain can be used to generate the sub-library of diverse Heavy chains.

Following selection for proper folding of the diverse domains by generic ligand binding of both sub-libraries, the "dummy" domain of one sub-library is replaced with the corresponding folding-selected domains from the other sub-library to generate a library of diverse, properly folded scFvs that can be selected for target binding. Beginning before April 4, 1997, this approach was undertaken; reduction to practice by successful identification of scFv antibodies that bind test antigens was achieved thereafter following continued diligent efforts, described below and documented in the enclosed notebook page copies.

A) The first notebook entry (A), which occurred before April 4, 1997, documents the construction of the vectors for this approach. Specifically, the notebook entries state:

"Construction of new vectors pCLEANV_H + pCLEANV_K for phage expression. These have dummy V_K and V_H respectively in IT linker in vector pH (no Tags, no TAG, no PHEN seq site)."

Notebook entry A documents the conception of the claimed invention and a beginning of the reduction to practice by preparing the necessary dummy Heavy and Light chain vectors before April 4, 1997. The reference to "no Tags, no TAG" means that the encoded vectors do not encode epitope or other heterologous polypeptide tags, and they do not encode TAG stop codons. Vectors DP-47 and DPK-9 supplied the V_H and V_K heavy and light chain antibody

frameworks, respectively. These V_H and V_K frameworks are also useful in non-scFv antibody formats.

B) *Notebook entry (B)*, shows the results of sequencing reactions, performed before April 4, 1997, to check the Heavy and Light chain dummy vectors. The entry documents the identification of VH2-4 as pCLEANV $_K$ and V $_K$ 1-5 as pCLEANV $_H$. Primary PCR amplifications for the library according to the invention were carried out on this date.

C) *Notebook entry (C)* documents the gel purification of the primary amplifications and the use of the isolated products for secondary amplifications to introduce diversity. The germline V gene segments DPK-9 (V_K) and DP-47 (V_H) were used as templates for PCR mutagenesis. The different sub-libraries were made to include variability that mimics the introduction of diversity *in vivo* – the “primary” sub-libraries have diversity introduced in the H2, H3 and L3 loops, mimicking germline or junctional diversity; the “somatic” sub-libraries have diversity introduced in H1, H3, L1 and the end of L3 loops, mimicking diversity introduced as a result of somatic hypermutation. Diversity was introduced through use of amplification primers with the diversity codons “NNK” or “DVT.” The “NNK” diversity codon encodes all 20 amino acids, including the TAG stop codon, but not the TGA or TAA stop codon. The “DVT” diversity codon encodes 22% serine and 11% tyrosine, asparagine, glycine, alanine, aspartate, threonine and cysteine, and closely mimics the distribution of amino acid residues in the antigen binding sites of natural human antibodies.

The differing mutagenic codon approaches produced four separate fragments for each of the NNK and DVT libraries: a “primary” V_H fragment (5A); a “primary” V_K fragment (6A), a “somatic” V_H fragment (5B); and a “somatic” V_K fragment (6B). Each of the amplified fragments was digested overnight with restriction enzymes for cloning.

Over the following days, the products of the overnight digestion were gel purified, and test ligations were set up using digested secondary amplification products and pCLEAN vector (See Notebook Entry D). Ligated DNAs were transformed into HB2151 *E. coli* cells. The results of the test ligations were used to set up main ligations for V_H and V_K libraries according

to the invention. The main ligations were transformed into HB2151 *E. coli* cells. Clones from the main ligations were assessed for insert.

D. The V_H and V_K libraries were then rescued to produce phage particles. *Notebook entry (D)* documents the generation of phage that display the molecules of the V_H and V_K libraries on their surfaces. The ligations from Entry C were electroporated into *E. coli* strain HB2151. The resulting phage titers for sub-libraries (5A, 5B, 6A, 6B) are shown at the bottom of the page of Notebook Entry D.

E. The resulting phage libraries were then selected against Generic Ligands. *Notebook entry (E)* shows selection of the V_H sub-libraries 5A and 5B for folding by binding to Protein A, and the selection of the V_K sub-libraries 6A and 6B by binding to Protein L.

Phage from each of the libraries were separately selected using immunotubes coated with 10 µg/ml of generic ligands Protein A and Protein L for the V_H and V_K libraries, respectively. High titres for all four selected libraries indicate successful selection for functional/folded members. This demonstrates selection for function/folding against Generic Ligands.

F. DNAs from the libraries selected for function/folding and those not selected for function/folding were then prepared and cut with appropriate restriction enzymes. Vector and V_H library selected for function/folding was test-ligated with insert comprising the V_K library selected for function/folding. Also, Vector and V_H library not selected for function/folding was test ligated with insert comprising V_K library not selected for function/folding.

Also at this time, an ELISA screen was established for selection for function/folding. The screen involves a sandwich ELISA of Protein A on a plate. To establish the screen, the plate is overlaid with supernatant of dummy V_H-V_K library member (6A, 6B) or V_H library member-dummy V_K (5A, 5B) or V_H library member-V_K library member (NNK A, NNK B). Detection with Protein L-Horse radish peroxidase (HRP) indicated functional/folded V_K library member, V_H library member, or paired V_H-V_K library members, respectively.

Also during this time, main library ligations were performed, combining vector and V_H library selected for function/folding with insert comprising V_K library selected for

function/folding (Libraries C and D). This shows selection against a Generic Ligand to create a subset, followed by combination with selected subset from the other chain. Main library ligations combining vector and VH library that had not been selected for function/folding were test ligated with insert comprising VK library not selected for function/folding (Libraries E and F). (See *Notebook entry F*). Resulting libraries were transformed into *E. coli*.

G. At this time, Library C had to be re-made due to a transformation failure (See *Notebook entry G*, top of page, stating "Library C remake").

H. Subsequent to the re-creation of Library C, clones from the VH-VK main library ligations (selected VH and VK) were tested for insert. VH-VK clones not selected against Generic Ligands were also tested for insert. *Notebook entry H*, 2 pages shows the results when clones from these libraries were expressed and tested in the ELISA function/folding screen. A high percentage of functional/folded clones was noted where there was pre-selection with the Generic Ligands compared with a low percentage in the absence of pre-selection with Generic Ligands. Specifically, *Notebook Entry H* shows that pre-selected Library C gives 81 of 96 positive and pre-selected Library D gives 91 of 96 positive, while non-selected Library E gives 34 of 96 positive and non-selected Library F gives 39 of 96 positive. It is also noted that the degree of binding is generally higher in the pre-selected clones. These data demonstrate that pre-selection with Generic Ligands enriches for functional/folded library members.

I. Subsequently, functional/folded and non-functional/folded clones from the screens were sequenced. All non-functional/folded clones sequenced had defects such as frame shifts or inappropriate stop codons that prevent function/folding.

Subsequently, new starting VH and VK libraries were test ligated into pCLEAN vectors and transformed into HB2151 *E. coli* cells.

At the same time, more functional/folded and non-functional/folded clones from the previous screen were sequenced. *Notebook entry I* (see lanes 1-20 in sequencing run NEW 18) shows the results of sequencing runs in which all folding/function-negative clones sequenced had defects (e.g., frame shift, TGA, TAG codons) that prevent function/folding. These results indicate that non-folding is not simply the result of non-expression. This is a demonstration that

selection with Generic Ligands selects for more than simply expression, and rather, selects for properly folded protein.

J. Subsequently, the new VH and VK libraries were rescued to produce phage particles. Protein A and Protein L were coated onto immunotubes and new VH and VK libraries according to the invention were selected for function/folding in the ELISA sandwich assay format. High titres for all four selected libraries indicate successful selection for functional/folded members.

Vector and the new VH library selected for function/folding was then test ligated with insert comprising the new VK library selected for function/folding.

New main library ligations combining vector and VH library selected for function/folding with insert comprising VK library selected for function/folding were then performed to establish Libraries G and H. Libraries G and H were transformed into *E. coli*. *Notebook entry J* shows resulting titres of libraries C, D, G and H (lower, right).

K. Subsequently, clones from the new main library were tested for insert (see *Notebook entry K*) and then expressed and tested in the function/folding ELISA screen. A high percentage of clones (72 of 96 with library G and 71 of 96 with Library H) was functional/folded where there was pre-selection with Generic Ligands, compared to a low percentage in the absence of pre-selection with Generic Ligands (see previous non-selected).

L. Phage resulting from folding/function pre-selected libraries were concurrently selected against Target Ligands. The Target Ligands included FITC-BSA, recombinant human leptin, thyroglobulin, BSA and hen egg lysozyme (HEL) – see *Notebook entry J*. Titres from the first round of selections are shown in the “r1 selections” table on *Notebook Entry J*.

M. Titres from a second round of selections against Target Ligands (“r2 selections”) are shown in *Notebook entry L*. Titres for a third round (“r3 selections”) are shown in *Notebook Entry M*.

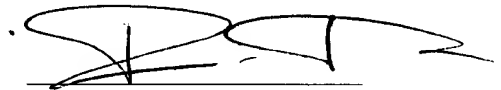
N. Target Ligand binding assays were then performed using clones from the third round of Target selection. *Notebook entry N* shows the results of target binding assays of monoclonal VH-VK pairings to the target ligands in assays using the immobilized target ligands and labeled

Generic Ligand for detection. Positive clones represent the successful selection of polypeptides that bind to Target and Generic Ligands. This represents a full reduction to practice of the claimed invention. This successful reduction to practice was the result of continuous diligent efforts from before the April 4, 1997 date of the Buechler et al. reference.

5. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

12 SEPT ' 2003

Date

A handwritten signature in black ink, appearing to read 'I. Tomlinson', written over a horizontal line.

Ian M. Tomlinson

EXHIBIT 1

TEBOOK ENTRY A

Construction of new vectors π pCLOON VH + pCLOON VK
for phage expression - these have dummy VK and VH
respectively in IT linker in vector pH (no tags, no TAG, no
pHEN seq, etc).

pH sequence checked using LMB3 - OK!

cut Sfi I / Nde I O/N + ligate 'INSERT'

24 colonies checked - all true

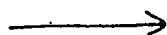
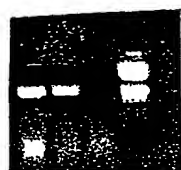
Take one of these cuts Nco I / Xho I + $Sall$ / Nde I

PCR DP-47 + DPK9 with FR1B primer + CDR3 primer for

dummy chains. (DP-47 good with Ph, DPK9 not - used Tag)

PCR prep these + re PCR with SF/B + OF/B respectively

(25 cycles)



cut amplified bands as above - ligate + transform into TG-1

Check ~~sequences~~ for insert (see next page)

VH screen LMB3 link seq

VK screen DPK9 FR1 seq, GUT fwd LMB2

BOTH LMB3 GUT fwd LMB2

(Mix up but VH2 + VK1 good - check 6x sequenced)

VH2-4 is good

sequences for VK1-3, 4, 5 not long enough

use other primers on next template.

ell

NOTEBOOK ENTRY B

Recheck of p CLEAN VECTORS

Now 16

WAS

	No errors	EMB3	linkseq	G3 fwd	rdseq ^{rc}	brkqtriseq
③ VH2-4	✓	1	6	11	16	
④ VK1-3	✓	2	7	12	17 Drms	21 Drms
⑤ VK1-4	✓	3	8	13	18 "	22 "
⑥ VK1-5	✓	4	9	14	19	23 "
PIT3		5	10	15	20	

VH2-4 is p CLEAN VK

~~VH2-4 is p CLEAN VK~~

VK1-5 is p CLEAN VH

PIT3 is GOOD!

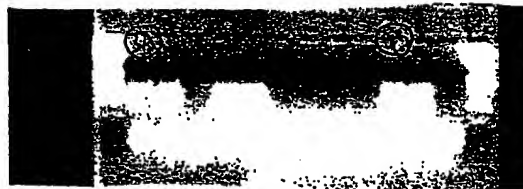
SYGAW library construction

Vectors 10 pcr's performed (conditions as NNE Ws)

30 cycles



25 cycles

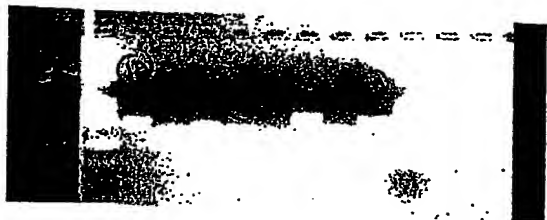


20 cycles

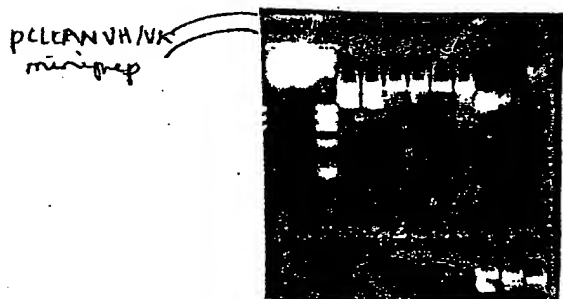


These gel purified & cleaned up. (1A, 2A, 3A, 4A → electroboration
then EtOH prt
5A, 7A P/C x 2 EtOH prt
6A, 8A gel electroboration)

NOTEBOOK ENTRY C



These mixed and amplified according to NNK protocol
(5ul used per PCR) (20 cycles with PR
1st 5 cycles without)



UB (5A used 1A+2A (1+2)
5B used 1B+2B (5+6)
VK (6A used 3A+4A (3+4)
6B used 3B+4B (7+8)

Test Ligations

NOTEBOOK ENTRY D

	V	INS	H ₂ O	cols	INS (0.4 screen)
1	1	5	0	130	23/24 20ul total
2	1	2	3	140	23/24 2 BUFP
3	1	0.5	4.5	156	20/24 0.5 LIGASE
4	1	0.1	4.9	99	15/24 11.5 H ₂ O
5	1	0	5	0	
				0	

Strom-Ligase

Master ligations use 1V: 21 100ul

LIG MADE IN HB2151

	SA	SB	6A	6B
SA	25	18	21	16
SB				
6A				
6B				

total n4 = 162.04

DNA Prepped from these 1ml glycerol → 40ml 2xTT
Prepped with Qiagen mid prep

Also inoculated 100μl into 100ml 2xTT AMP GLU
PA/PL coated O/N (10μg/ml in PBS) for selection.

(14.75 ml total) STANDARD WASH CONDITIONS 20x20x.

	COLS ON 10K	TITRE (IN 14.75mls) x 2.45 x 10 ⁶
5A	460	1.36 x 10 ⁹
5B	541	1.60 x 10 ⁹
6A	518	1.53 x 10 ⁹
6B	304	8.97 x 10 ⁸

cells were -ve.

DNA Prepped with Qiagen mid prep kit - resuspended in.

12.5μl TOTAL
10μl in 12.5μl

40μl of this into 80μl reaction

8 Sal I BUFF
2 Sal E
2 Not I
28 H₂O
40 ONA

Also unselected cells

Trial ligations SYG A, SYG AC

20μl

	SYG	AV	GA I	1 LIGASE + 14 H ₂ O	16 H ₂ O
SYG AC	1	0.5	0	1/5 2.5	30x5
	2	0.5	0.1	1/4 2.6	30x5
	3	0.5	0.5	1/2 2	30x5
SYG A	4	0.5	2.5	1/4 2.5	100x5
	5	1	0	1/5 2.5	100x5
	6	1	0.1	1/4 2.6	100x5
	7	1	0.5	1/2 2	100x5

Use 1:3
More COLS ON
SYG A THAN
SYG AC!

B1G-L1B ligation 100 μ l

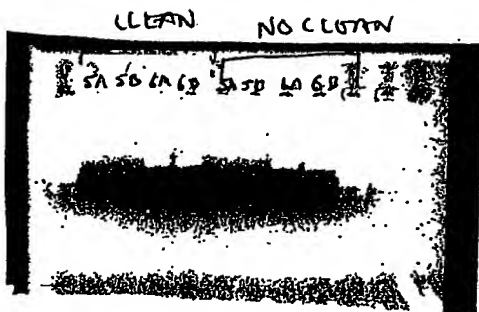
10 BUPT

10 Vector: (.5A cuts)

20 INSERT (6A cent)

4 LIGASE

56 H₂O



After gel extraction:

8x BLASTS each library (65ul) Add 13ul 2xT7

Pool cell (1.6mls) \rightarrow (160 μ l \rightarrow 2ml) \rightarrow (200 μ l \rightarrow 2ml) \rightarrow (200 μ l \rightarrow 2ml)

LIB	10	10 ²	10 ³	
C A CLEAN	255	8.16 × 10 ⁷	8.16 × 10 ⁶	8.16 × 10 ⁵
D B CLEAN	744	2.38 × 10 ⁸	2.38 × 10 ⁷	2.38 × 10 ⁶
E A	781	2.5 × 10 ⁸	2.5 × 10 ⁷	2.5 × 10 ⁶
F B	706	2.26 × 10 ⁸	2.26 × 10 ⁷	2.26 × 10 ⁶

AGAROSU

DRUPPUD

OFF PLATE

- 15 - ve

pH has 30 on 10^2 1.2×10^9

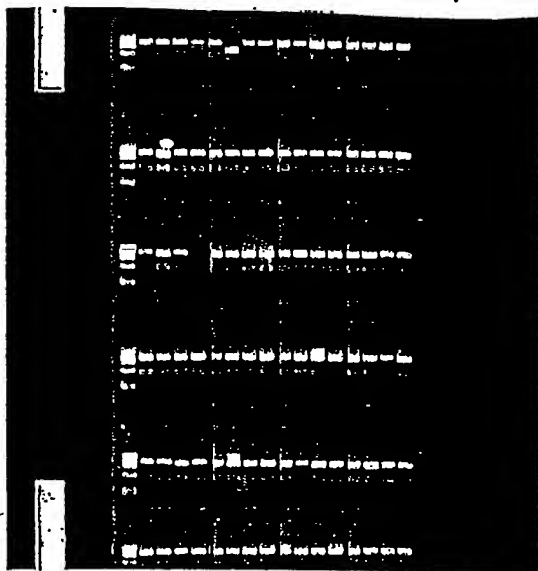
↓
CONGRAT!

Use V_{CO}

of 102

— 170725 com m/200000

reder 20x BLASTS w/ new/old ligations.



LIB c remove (A cleaned S7.6 (DVT))

COLS ON 10⁴ BSA/BSA (NBT, 10, 10³, 10⁵) 10 S124 10³ S124 10⁵ S124
 1024 5.57 x 10⁸ 5.57 x 10⁷ 5.57 x 10⁶ 5.57 x 10⁵ 444

THIS IS C-2. -ve is -ve

C-2, b, E, F. DNA Pepped + alk Nco / Not (NEB3 + BSA)
 IMP gel → Gel electrophoresis → ligate into pAB1.

TEST selection with C2, D2, E2, F2, C3, D3, E3, F3
 Make phage, select on 1 minute tubes - (25 µg/ml NIPBSA)

MOLECULAR DEVICES
New Data (Plate)

DATA FILE: c/d/e/f unset
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 20.60°C

AUTOMIX: OFF

CALIBRATION: ON
 SET TEMP: OFF

1024 (µg/ml) C2 D2 E2 F2 - Optical Density

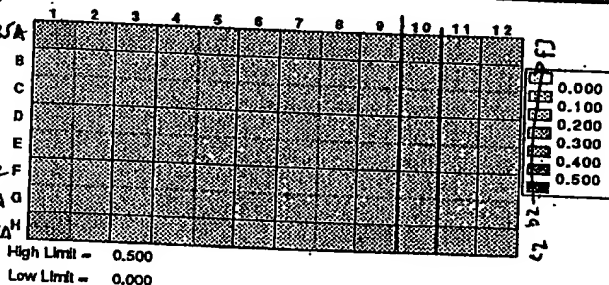
	1	2	3	4	5	6	7	8	9	10	11	12
A	0.214	0.124	0.181	0.126	0.085	0.082	0.111	0.131	0.033	0.140	0.049	0.166
B	0.032	0.027	0.018	0.024	0.019	0.015	0.018	0.032	0.044	0.039	0.047	0.019
C	0.073	0.104	0.042	0.046	0.040	0.041	0.021	0.014	0.019	0.023	0.081	0.076
D	0.149	0.122	0.050	0.084	0.074	0.077	0.111	0.014	0.087	0.050	0.029	0.020
E	0.077	0.037	0.013	0.009	0.010	0.040	0.011	0.028	0.024	0.023	0.044	0.098
F	0.014	0.021	0.014	0.028	0.013	0.013	0.012	0.019	0.017	0.018	0.018	0.019
G	0.054	0.010	0.020	0.030	0.011	0.009	0.041	0.084	0.018	0.026	0.054	0.082
H	0.229	0.175	0.037	0.013	0.112	0.082	0.084	0.130	0.221	0.120	0.137	0.119

MOLECULAR DEVICES
Gray Scale (Plate)

DATA FILE: c/d/e/f unset
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 20.60°C

AUTOMIX: OFF

CALIBRATION: ON
 SET TEMP: OFF



10 µl unselected phage against
 No real evidence for specific binding

For test selection
 cols on 10²

C2	247	7.41 x 10 ⁶
D2	212	6.36 x 10 ⁶
E2	165	4.45 x 10 ⁶
F2	53	1.59 x 10 ⁶

-ve is -ve

cols on 10² 30,000

C3	92	2.76 x 10 ⁶
D3	243	7.29 x 10 ⁶
E3	158	4.74 x 10 ⁶
F3	154	4.62 x 10 ⁶

Make phage do ELISA

DEF-ives 0 SCORING 1 ON 1 (depths) H 1151 50m
 2.1 ON WITH THE B...
 1:5000 PA-HRP

DATA FILE: C1st
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 21.70°C
 AUTOMIX: OFF
 CALIBRATION: ON
 SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.191	1.280	1.324	1.413	1.433	0.413	0.823	1.500	1.472	0.341	1.372	0.045
B	0.494	1.392	1.378	1.422	1.228	1.435	0.759	0.218	1.001	1.308	1.288	1.294
C	0.03	1.278	0.278	1.437	0.148	0.552	1.480	1.343	1.442	1.348	1.320	0.368
D	0.182	0.03	0.02	1.124	1.250	1.478	1.270	1.283	0.110	1.285	0.999	0.044
E	0.083	0.032	1.481	0.045	0.648	1.145	1.318	0.027	1.291	1.244	1.143	1.460
F	1.380	0.297	0.198	1.042	0.032	1.648	1.333	1.401	0.844	1.200	1.384	1.474
G	1.138	0.239	1.339	1.220	0.435	0.04	1.481	1.012	1.288	0.044	1.375	1.228
H	1.293	1.106	0.030	1.177	1.308	1.415	1.414	1.273	1.438	1.384	1.289	1.330

DATA FILE: C2nd
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 21.90°C
 AUTOMIX: OFF
 CALIBRATION: ON
 SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.230	1.280	1.368	1.361	1.378	0.195	1.084	1.498	1.521	0.228	1.488	0.03
B	0.588	1.291	1.284	1.305	1.147	1.325	0.032	0.287	1.172	1.261	1.359	1.423
C	0.031	1.240	0.340	1.291	0.217	0.053	1.418	1.383	1.491	1.321	1.383	0.800
D	0.288	0.049	0.032	1.007	1.203	1.431	1.078	1.298	0.400	1.245	1.028	0.054
E	0.108	0.03	1.405	0.004	0.965	1.289	1.319	0.031	1.422	1.403	1.340	1.580
F	1.401	0.381	0.258	1.068	0.041	1.611	1.308	1.469	1.115	1.420	1.620	1.475
G	1.157	0.381	1.288	1.295	0.455	0.03	1.498	1.127	1.387	0.073	1.448	1.401
H	1.261	1.132	0.044	1.212	1.238	1.305	1.259	1.117	1.388	1.288	1.279	1.359

DATA FILE: D1st
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.00°C
 AUTOMIX: OFF
 CALIBRATION: ON
 SET TEMP: OFF

Optical Density

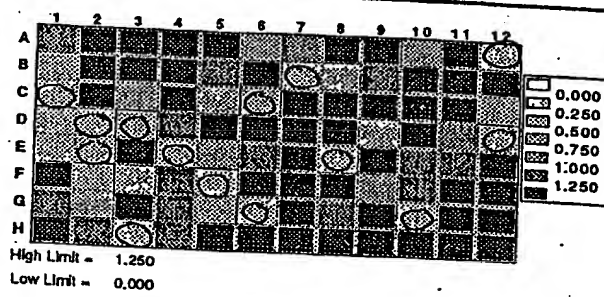
	1	2	3	4	5	6	7	8	9	10	11	12
A	1.923	1.834	1.678	1.447	1.356	1.492	1.437	1.320	1.291	1.458	1.412	1.408
B	0.263	1.494	0.910	1.342	1.626	1.379	1.328	1.418	1.402	1.410	1.411	1.382
C	1.384	0.883	0.027	1.455	1.539	1.305	1.429	1.478	1.301	0.027	1.486	1.485
D	1.610	1.522	1.641	0.027	1.418	1.447	0.382	1.473	1.508	1.400	0.920	1.385
E	1.574	1.480	1.438	1.318	1.489	0.840	1.442	1.383	1.312	1.001	1.352	1.325
F	1.613	1.459	0.027	1.412	1.307	1.509	1.284	1.407	1.428	1.449	1.308	1.271
G	1.278	1.430	1.488	1.170	1.382	1.501	1.335	1.437	1.438	1.474	1.224	1.402
H	1.582	1.355	1.584	1.474	1.448	1.331	1.518	1.115	1.358	1.338	1.369	1.388

DATA FILE: D2nd
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.00°C
 AUTOMIX: OFF
 CALIBRATION: ON
 SET TEMP: OFF

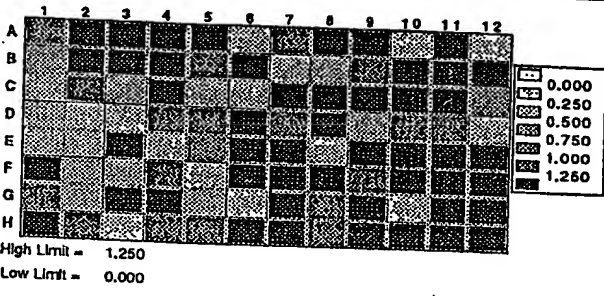
Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	1.284	1.224	1.303	1.305	0.928	1.283	1.245	1.181	1.078	0.987	1.283	1.168
B	0.053	1.248	0.692	1.183	1.217	0.820	0.715	1.148	1.233	1.071	1.248	1.148
C	0.882	0.837	0.03	1.428	1.355	1.206	1.218	1.213	1.289	0.03	1.383	1.221
D	1.404	1.424	1.338	0.03	1.297	1.298	0.587	1.210	1.427	1.091	1.160	1.148
E	1.282	1.310	1.211	1.375	1.318	0.748	1.288	1.042	0.582	1.142	1.164	1.199
F	1.282	1.093	0.019	1.353	1.184	1.338	0.989	1.251	1.288	1.238	1.228	1.036
G	0.818	1.177	1.257	1.090	1.009	1.237	1.123	0.824	1.132	1.277	1.166	1.139
H	1.219	1.205	1.509	1.355	1.328	1.187	1.388	1.078	1.378	1.230	1.028	1.285

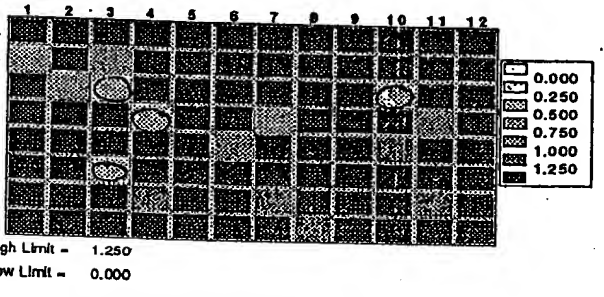
DATA FILE: C1st
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 21.70°C
 AUTOMIX: OFF
 CALIBRATION: ON
 SET TEMP: OFF



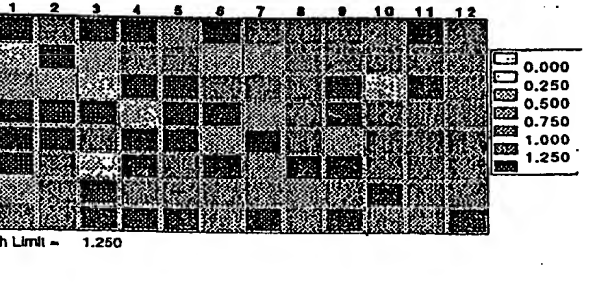
DATA FILE: C2nd
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 21.90°C
 AUTOMIX: OFF
 CALIBRATION: ON
 SET TEMP: OFF



DATA FILE: D1st
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.00°C
 AUTOMIX: OFF
 CALIBRATION: ON
 SET TEMP: OFF



DATA FILE: D2nd
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.00°C
 AUTOMIX: OFF
 CALIBRATION: ON
 SET TEMP: OFF



DATA FILE: E 1st
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.10°C

AUTOMIX: OFF

CALIBRATION: ON
 SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.037	0.021	0.031	0.030	0.030	0.030	0.078	0.100	0.091	0.133	0.407	0.067
B	1.252	0.205	0.020	0.03	0.02	1.558	0.180	0.020	0.747	1.061	0.03	1.439
C	1.678	0.582	0.820	0.02	0.05	0.02	0.03	0.00	0.02	1.489	1.393	0.03
D	0.030	0.027	0.04	0.04	0.02	0.02	0.02	0.02	0.100	0.04	0.948	0.03
E	0.017	0.000	0.163	0.070	0.023	1.204	0.057	0.02	0.02	0.02	0.02	1.188
F	0.872	1.306	0.085	0.02	0.02	0.030	0.150	0.067	0.030	0.840	0.037	
G	0.073	0.758	0.12	1.418	0.603	1.448	0.887	0.289	1.314	0.11	0.027	0.484
H	0.602	0.057	0.04	1.477	0.041	1.183	0.019	1.265	0.020	0.07	0.020	0.130

DATA FILE: E 2nd
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.10°C

AUTOMIX: OFF

CALIBRATION: ON
 SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.035	0.020	0.101	0.02	0.030	0.258	0.03	0.047	0.081	0.108	0.087	0.03
B	1.259	0.034	0.038	0.020	0.020	1.351	0.121	0.020	0.714	1.064	0.020	1.238
C	1.542	0.874	0.123	0.03	0.04	0.03	0.03	0.02	1.338	1.372	0.03	
D	0.030	0.020	0.041	0.04	0.02	0.03	0.020	0.138	0.074	0.04	0.768	0.04
E	0.047	0.058	0.178	0.04	0.032	1.278	0.070	0.03	0.069	0.031	0.085	1.233
F	0.871	1.228	0.03	0.02	0.027	0.03	0.225	0.11	0.020	0.02	0.642	0.030
G	0.030	0.446	0.030	1.358	0.173	1.241	0.033	0.108	1.004	0.05	0.024	0.034
H	0.307	0.558	0.037	1.375	0.047	0.953	0.025	1.038	0.027	0.03	0.027	0.039

DATA FILE: F 1st
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.10°C

AUTOMIX: OFF

CALIBRATION: ON
 SET TEMP: OFF

Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.310	0.042	1.148	0.199	1.430	0.108	0.061	1.099	0.03	0.131	0.086	0.247
B	0.310	0.042	0.045	0.030	0.030	0.213	1.125	1.128	0.03	0.03	0.238	1.449
C	0.030	1.481	0.020	0.020	1.417	1.459	0.141	1.488	0.02	0.03	0.450	1.871
D	1.206	0.04	0.020	1.509	0.045	0.280	0.020	0.182	0.228	0.020	0.330	0.020
E	0.030	0.071	1.183	0.03	0.030	0.045	1.349	0.020	0.123	0.386	1.327	0.208
F	0.04	0.03	0.03	0.030	0.030	0.04	1.377	0.383	0.020	0.027	0.04	0.02
G	1.523	0.047	1.289	0.03	0.045	1.372	1.305	0.020	0.030	0.020	0.03	0.02
H	0.03	0.070	0.03	0.03	0.382	0.020	0.112	0.02	0.08	0.378	0.138	1.493

DATA FILE: F 2nd
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.10°C

AUTOMIX: OFF

CALIBRATION: ON
 SET TEMP: OFF

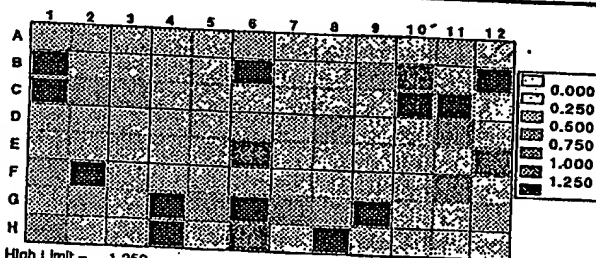
Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.817	0.059	0.493	0.04	1.393	0.578	0.03	0.741	0.02	0.189	0.053	0.181
B	0.015	0.01	0.03	0.02	0.03	0.125	0.798	0.03	0.04	0.03	0.122	1.210
C	0.04	1.219	0.04	0.03	1.317	1.309	0.089	1.343	0.03	0.04	0.548	1.491
D	0.797	0.03	0.03	1.248	0.037	0.188	0.03	0.239	0.173	0.020	0.192	0.07
E	0.04	0.04	1.039	0.03	0.03	0.04	1.287	0.03	0.03	0.188	1.146	0.193
F	0.03	0.03	0.034	0.03	0.03	0.03	1.240	0.284	0.034	0.031	0.034	0.02
G	1.174	0.040	0.888	0.03	0.03	1.012	1.070	0.020	0.034	0.021	0.021	0.02
H	0.03	0.04	0.03	0.03	0.108	0.037	0.03	0.03	0.04	0.118	0.053	1.259

DATA FILE: E 1st
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.10°C

AUTOMIX: OFF

CALIBRATION: ON
 SET TEMP: OFF

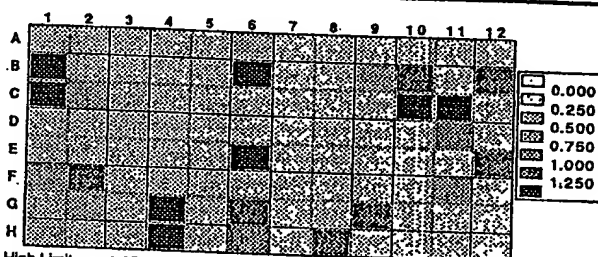


High Limit = 1.250
 Low Limit = 0.000

DATA FILE: E 2nd
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.10°C

AUTOMIX: OFF

CALIBRATION: ON
 SET TEMP: OFF

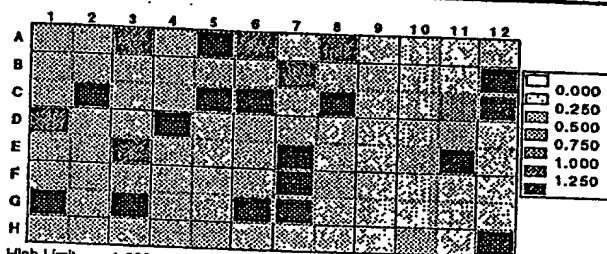


High Limit = 1.250
 Low Limit = 0.000

DATA FILE: F 1st
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.10°C

AUTOMIX: OFF

CALIBRATION: ON
 SET TEMP: OFF

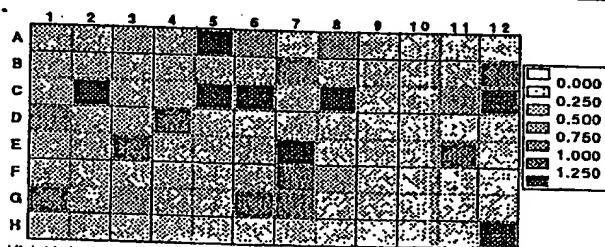


High Limit = 1.250
 Low Limit = 0.000

DATA FILE: F 2nd
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.10°C

AUTOMIX: OFF

CALIBRATION: ON
 SET TEMP: OFF



High Limit = 1.250
 Low Limit = 0.000

NOTEBOOK ENTRY H CONTINUED

SEQUENCING (NEW 18)

	VH	L	VK	L	Recon	DIV connect	VH	VK
NNKA + E1	linkseger		LM82	17	21		✓	✓
VNKA - E2	"	2	"	18	22 FS IN VK CDR3		✓	✓
NNKA + A2	"	"	"	19	23 FS IN VA PR 4 → still give 3 for CLISA		✓	✓
NNKB - A1	"	4	"	20	24 FS IN VA PR 4		✓	✓
C + A1	"	5	"	21	25		✓	✓
C - B7	"	6	"	22	26 FS IN VA CDR 2		✓	✓
b + A1	"	7	"	23	27		✓	✓
b - C7	"	8	"	24	28 FS IN VA CDR 1		✓	✓
E + B1	"	9	"	25	29		✓	✓
E - A2	"	10	"	26	30 FS IN VA CDR 2		✓	✓
F + A3	"	11	"	27	31		✓	✓
F - A2	"	12	"	28	32 FS IN VA PR 1		✓	✓
D3 NIP-BSAT2-08	"	13	"	29	33 GLLIB 11		X	✓
r3 - B8	"	14	"	30	34 KINTAL ROVN 35 or V11?		✓	✓
r3 - D11	"	15	"	31	35		✓	✓
r3 - D12	"	16	"	32	36		✓	✓
PROG1 BIP3 BIP A1	"				37			
M1	"				38			
C2 NIP-BSAT3-A5	linkseger			35				
C3 NIP-BSAT3-B3	"			36				

N.D. TEMPLATE NNKA B A2 is true but has FS IN VA PR 4
THIS WILL BE ELIMINATED BY PHOTOCOPY!

Selections Part II

	STOCK CONC	FINAL CONC	USE IN 3ml (PBS)
FITC-BSA (WGA)	5.2mg/ml	20µg/ml	10.53µl
LEPTIN (rh)	3.3mg/ml	50µg/ml	4.545µl
THYROGLOBULIN	1mg/ml	100µg/ml	1.50µl
BSA	10mg/ml	100µg/ml	30µl
HEL	18.4mg/ml	500µg/ml	81.52µl

LIBS C, D, G & H Prepared as follows
 400µl (G/H) or 200µl (C/D) of glycerol stocks → 250ml 2xTYMP 1% Glu 100µg/ml

Growth till OD = 0.5 Add 50µl VCS stock to 50ml culture
 Incubate 20 minutes at 37°C (water bath) Spin & resuspend in
 250ml 2xTY, 100µg/ml AMP, 50µg/ml KAN, 0.2% Glu. Grow ON at 30°C.

200µl of this spin → super for PEG ppt x2 → 10ml PBS

0.5ml C added to 0.5ml G select x5

0.5ml D added to 0.5ml H select x5

	TITERS ON 10 ⁶ cells	INPUT PHAGE/ml
C	79	1.58×10^{13}
D	91	1.82×10^{13}
G	45	9.00×10^{12}
H	87	1.74×10^{13}

selections

colony number

TITERS

CG INPUT = $1.24 \times 10^{13}/ml$

DH INPUT = $1.78 \times 10^{13}/ml$

CG SIZE = 1.48×10^9

DH SIZE = 1.24×10^9

[EQUIV SIZES COMP TO AE 146x11
 infected & clean. BR 6.39x1

10 CG	240	7.06×10^6
11 CG	132	3.88×10^6
12 CG	260	7.64×10^6
13 CG	300	8.82×10^6
14 CG	74	2.18×10^6
10 DH	234	6.88×10^6
11 DH	254	7.47×10^6
12 DH	146	4.29×10^6
13 DH	102	3.00×10^6
14 DH	68	2.00×10^6

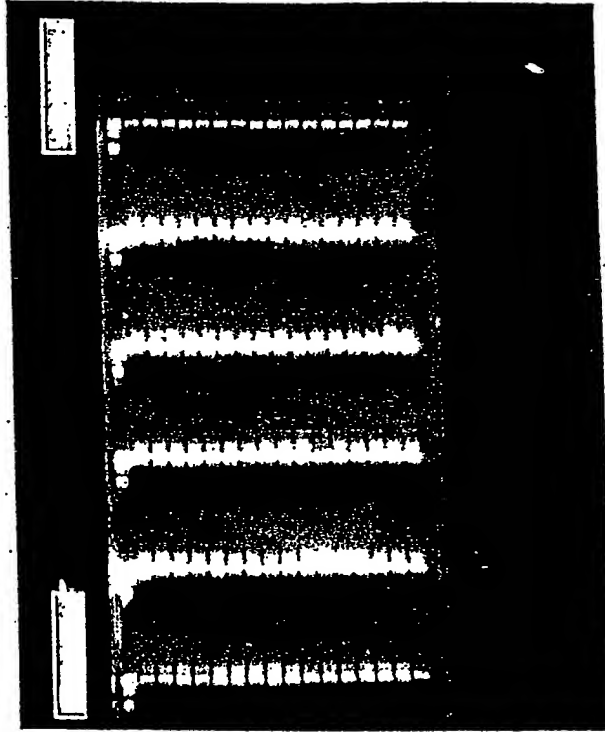
NOTEBOOK ENTRY K

PA/PA assay on tubes C+H (pAB1 HB2LS1 redones)

PCR

LIB G

LIB H



DATA FILE: G1st
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.30°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF

DATA FILE: G1st
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.30°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF

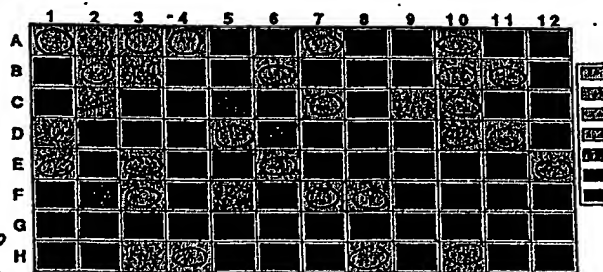
Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.088	0.458	0.100	0.108	2.694	1.692	0.097	1.057	2.451	0.100	2.324	2.192
B	2.360	0.099	0.503	1.942	1.379	0.089	2.059	2.280	2.099	0.101	0.101	2.108
C	2.351	0.471	1.182	2.287	0.938	1.994	0.100	2.192	0.151	0.109	2.044	2.251
D	0.434	2.049	2.448	2.088	0.120	0.995	2.397	2.439	1.981	0.108	0.110	2.417
E	0.109	2.247	0.116	1.954	2.414	0.402	2.213	2.282	2.330	1.650	2.335	0.117
F	1.840	1.011	0.127	2.143	0.273	2.181	0.102	0.096	2.222	2.164	2.182	2.573
G	2.097	1.957	1.790	2.310	2.315	2.319	2.291	2.464	1.955	2.481	2.569	2.187
H	2.589	1.750	0.387	0.107	2.443	1.870	1.851	0.114	2.304	0.140	1.319	2.393

DATA FILE: G2nd
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.50°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF

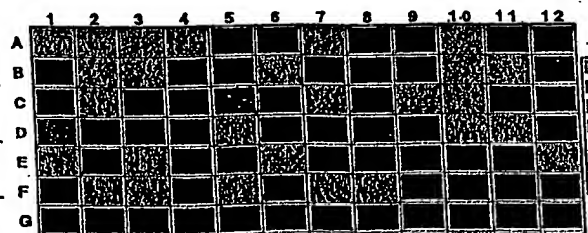
Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	0.098	0.273	0.088	0.129	2.599	1.607	0.097	1.115	2.375	0.093	2.110	2.184
B	2.112	0.108	0.475	2.022	1.557	0.098	1.984	2.120	2.345	0.100	0.098	2.308
C	2.244	0.499	1.177	2.246	0.869	1.987	0.095	2.107	0.163	0.103	2.185	2.141
D	0.699	2.153	2.271	1.940	0.103	1.147	2.294	2.363	1.909	0.095	0.105	2.309
E	0.151	2.055	0.136	1.699	2.254	0.108	2.157	2.185	2.323	1.793	2.207	0.113
F	1.767	0.782	0.159	2.019	0.277	2.100	0.111	0.112	2.121	2.170	2.023	2.437
G	2.589	1.750	0.387	0.107	2.443	1.870	1.851	0.114	2.304	0.140	1.319	2.393



High Limit = 1.250
Low Limit = 0.100

DATA FILE: G2nd
DESCRIPTION:
PROTOCOL:
DESCRIPTION:
MODE: Dual End.
WAVELENGTH: 450-650
MEAN TEMP: 22.50°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF



DATA FILE: H 1st
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.80°C

AUTOMIX: OFF

CALIBRATION: ON
 SET TEMP: OFF

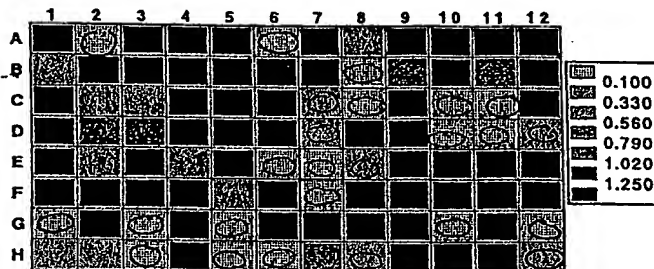
Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
I	1.958	0.079	1.458	2.049	1.906	0.088	2.042	0.251	2.261	2.027	2.089	2.265
J	0.317	1.248	1.756	1.947	2.070	2.157	1.976	0.086	0.995	1.924	0.968	2.019
K	1.627	0.319	0.269	1.013	1.448	1.901	0.101	0.085	2.037	0.090	0.089	1.691
L	2.166	1.002	0.976	1.864	2.019	2.047	0.137	1.804	2.121	0.077	0.080	0.100
E	2.530	0.164	2.148	0.155	2.010	0.091	0.081	0.138	2.284	1.855	1.118	2.007
F	1.441	2.118	2.038	2.110	0.655	1.870	0.097	2.134	2.140	1.650	1.478	1.963
G	0.084	1.938	0.084	1.341	0.092	1.892	2.400	2.178	2.084	0.083	2.393	0.089
H	0.194	0.421	0.083	1.908	0.091	0.093	0.928	0.121	1.708	2.098	1.614	0.130

DATA FILE: H 1st
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.80°C

AUTOMIX: OFF

CALIBRATION: ON
 SET TEMP: OFF



High Limit = 1.250

Low Limit = 0.100

DATA FILE: H 2nd
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.80°C

AUTOMIX: OFF

CALIBRATION: ON
 SET TEMP: OFF

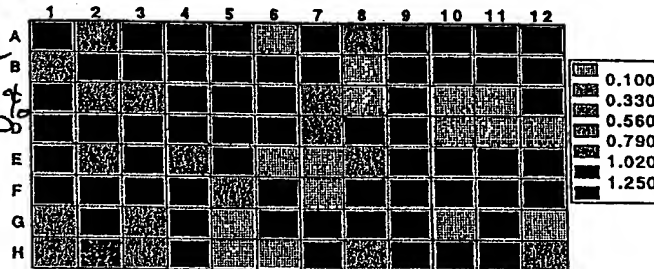
Optical Density

	1	2	3	4	5	6	7	8	9	10	11	12
A	2.003	0.103	1.598	2.140	1.920	0.094	2.058	0.459	2.194	1.991	1.988	2.359
B	0.362	1.588	1.984	2.083	2.111	2.220	1.965	0.098	1.768	2.159	1.140	2.020
C	1.734	0.461	0.558	1.993	1.701	2.041	0.116	0.087	2.023	0.085	0.082	1.962
D	2.108	1.576	1.623	2.225	2.185	2.115	0.186	1.775	2.177	0.074	0.079	0.087
E	2.545	0.347	2.120	0.160	2.129	0.089	0.091	0.118	2.293	2.098	1.244	2.108
F	1.874	2.301	2.085	2.247	0.763	2.099	0.088	2.058	2.219	1.935	1.637	2.090
G	0.105	2.048	0.100	1.644	0.098	2.188	2.418	2.137	2.218	0.074	2.383	0.087
H	0.344	0.858	0.107	2.175	0.092	0.090	1.248	0.131	1.930	2.140	1.589	0.151

DATA FILE: H 2nd
 DESCRIPTION:
 PROTOCOL:
 DESCRIPTION:
 MODE: Dual End.
 WAVELENGTH: 450-650
 MEAN TEMP: 22.80°C

AUTOMIX: OFF

CALIBRATION: ON
 SET TEMP: OFF



High Limit = 1.250

Low Limit = 0.100

Score < 0.150 ON ~~both~~ ^{either} -ve
 > 0.150 ON BOTH +ve

52 SELECTIONS

cols. on 102 (5-1000) ^{size} 512E

10CG 84 6.72×10^5

11CG 506 4.05×10^6

12CG 3 2.40×10^4

13CG 23 1.84×10^5

14CG 116 9.28×10^5

10BH 124 9.92×10^5

11BH 346 2.77×10^6

12BH 55 4.40×10^5

13BH 206 1.65×10^6

14BH 562 4.50×10^6

seems v. low (?) redo? if r3
 poly phase -ve

NOTEBOOK ENTRY M

r3

	cells $\times 10^2$	SIZE	ANTIGEN
10 CG	4	3.2×10^4	FITC-BSA
1 CG	159	1.77×10^6	LEPTIN
2 CG	~800	6.40×10^6	THYROGLOBULIN
13 CG	45 ($\times 10^4$)	3.60×10^7	BSA
14 CG	44	3.52×10^5	HEL
10 DH	9	7.20×10^4	FITC-BSA
11 DH	14	1.12×10^5	LEPTIN
12 DH	24	1.92×10^5	THYROGLOBULIN
13 DH	28 ($\times 10^4$)	2.24×10^7	BSA
14 DH	1	8.00×10^3	HEL

All r3 phage (except 11 CG had on awful lot of phage - possible

BSA binder from CG + old titer \rightarrow no evidence (yet!) (or: ^{contaminated} ^{germ} ^{growth})

binder from other selections

Make r3 phage + do 24 x each r3 monoclonals

NOTEBOOK ENTRY N

ISAS

FTC/leptin poly

Dual End. 450-650
20.30°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF

Optical Density

	4	5	6	7	8	9	10	11	12
140	0.210	0.069	0.053	0.211	0.164	0.099	0.288	0.217	0.008
17	0.080	0.062	0.035	0.063	0.055	0.080	0.287	0.120	0.008
180	0.080	0.050	0.059	0.068	0.067	0.093	0.102	0.208	0.007
72	0.138	0.073	1.722	0.074	0.098	0.139	0.105	0.178	0.005
152	0.019	0.014	0.020	0.038	0.058	0.063	0.117	0.069	0.006
168	0.050	0.100	0.074	0.035	0.068	0.048	0.012	0.038	0.005
77	0.097	0.097	0.087	0.082	0.125	0.042	0.139	0.081	0.007
13	0.080	0.077	0.058	0.121	0.167	0.128	0.167	0.162	0.008

FTC-BSA

LEPTIN

FTC/leptin mono

Dual End. 50-650
0.70°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF

Optical Density

	4	5	6	7	8	9	10	11	12
11	0.051	0.042	0.087	0.054	0.080	0.058	1.518	0.078	0.100
12	0.048	0.043	0.059	0.053	0.050	0.045	0.040	0.131	0.082
16	1.413	1.602	0.044	1.453	1.382	1.552	0.058	0.080	1.629
17	1.547	1.639	1.405	1.494	1.341	1.497	0.087	0.051	1.628
14	0.053	0.024	0.164	0.020	0.021	0.065	0.032	0.017	0.060
14	0.040	0.071	0.084	0.042	0.185	0.082	0.081	0.034	0.076
18	0.079	0.068	0.097	0.078	0.081	0.081	0.078	0.060	0.107
18	0.108	0.100	0.071	0.144	0.072	0.057	0.075	0.083	0.077

FTC-CC
DP

LEPTIN-CC

E: Thy/BSA poly

N:
N:
N:
E: Dual End. 450-650
H: 20.50°C
P:
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF

Optical Density

3	4	5	6	7	8	9	10	11	12
0.071	0.018	0.305	0.033	0.099	0.069	0.065	0.027	0.028	0.007
0.172	0.024	0.030	0.032	0.085	0.086	0.087	0.053	0.048	0.007
0.075	0.062	0.018	0.067	0.104	0.102	0.070	0.024	0.034	0.008
1.190	0.043	0.017	0.032	0.057	0.157	0.187	0.034	0.020	0.005
0.024	0.049	0.053	0.051	0.117	0.028	0.035	0.037	0.055	0.004
0.075	0.074	0.047	0.034	0.058	0.087	0.087	0.099	0.127	0.007
0.054	0.354	0.071	0.043	0.051	0.090	0.310	0.113	0.182	0.007
0.090	1.367	0.181	0.420	0.035	0.135	1.356	1.027	0.138	0.007

THY

BSA

Thy/BSA mono

Dual End. 450-650
20.80°C
AUTOMIX: OFF
CALIBRATION: ON
SET TEMP: OFF

Optical Density										
	3	4	5	6	7	8	9	10	11	12
130	0.198	0.512	0.841	0.328	0.371	0.142	0.623	0.387	0.202	
510	0.698	0.057	0.474	0.521	0.678	0.454	0.400	0.058	0.718	
558	0.575	0.044	0.087	0.044	0.309	0.082	0.071	0.094	0.093	
088	0.100	0.045	0.078	0.084	0.098	0.054	0.055	0.074	0.830	
115	1.210	1.150	1.185	0.087	1.207	0.708	1.182	1.308	1.598	

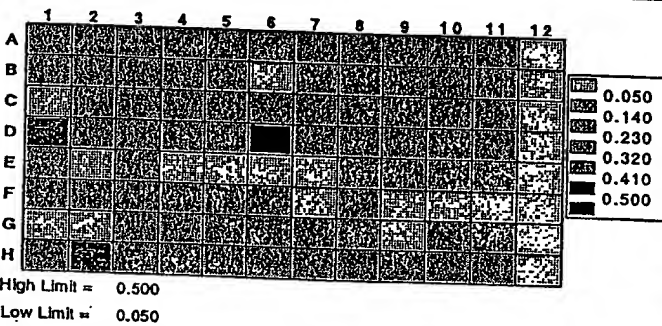
THY

BSA

DATA FILE: FTC/leptin poly
DESCRIPTION: PROTOCOL:
DESCRIPTION: MODE: Dual End. 450-650
WAVELENGTH: 20.30°C
MEAN TEMP:

AUTOMIX: OFF

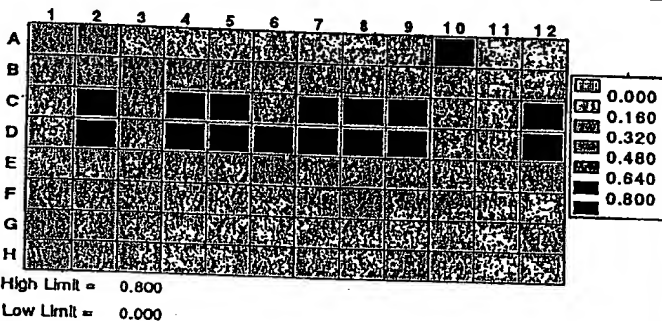
CALIBRATION: ON
SET TEMP: OFF



DATA FILE: FTC/leptin mono
DESCRIPTION: PROTOCOL:
DESCRIPTION: MODE: Dual End. 450-650
WAVELENGTH: 20.70°C
MEAN TEMP:

AUTOMIX: OFF

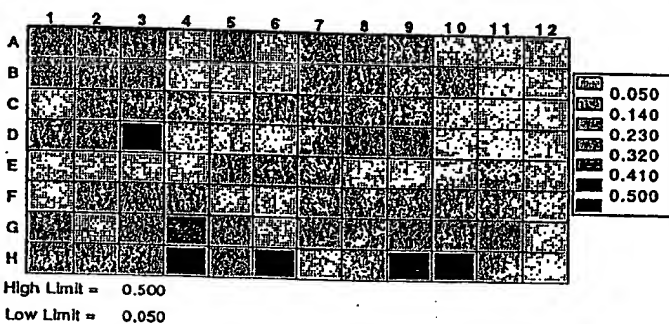
CALIBRATION: ON
SET TEMP: OFF



DATA FILE: Thy/BSA poly
DESCRIPTION: PROTOCOL:
DESCRIPTION: MODE: Dual End. 450-650
WAVELENGTH: 20.50°C
MEAN TEMP:

AUTOMIX: OFF

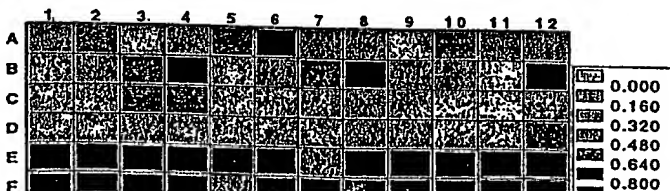
CALIBRATION: ON
SET TEMP: OFF

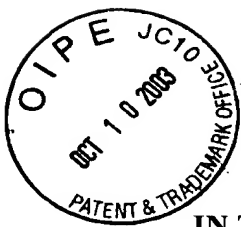


DATA FILE: Thy/BSA mono
DESCRIPTION: PROTOCOL:
DESCRIPTION: MODE: Dual End. 450-650
WAVELENGTH: 20.80°C
MEAN TEMP:

AUTOMIX: OFF

CALIBRATION: ON
SET TEMP: OFF





Atty. Docket No.: 8039/1070

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of:	Tomlinson, et al.	Examiner:	Ponnaluri, P.
Serial No.:	09/511,939		
Filed:	February 24, 2000	Group Art Unit:	1639
Entitled:	Method to Screen Phage Display Libraries with Different Ligands		
		Conf. No.:	5170

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF GREG P. WINTER UNDER 37 C.F.R. §1.131

I declare:

1. I, Greg P. Winter, am an inventor of the invention claimed in the above-noted U.S. Patent application.
2. I have read and understood the Office Action mailed July 11, 2003 and have read and understood the cited reference, U.S. Patent No. 6,057,098 (the "'098 patent," issued to Buechler et al. on May 2, 2000 from an application filed April 4, 1997). I understand that the Examiner has cited the '098 patent as a novelty reference over claims 33-41 and 44-52.

The '098 patent is cited as teaching methods of producing a multivalent polypeptide display library comprising a library of phage representing tagged fusion proteins. The Examiner states that the tag can be any polypeptide with a known receptor showing high binding specificity for the tag (referring to column 7, lines 10-11). The Examiner further asserts that Buechler et al. teaches contacting the library with a receptor (which the Examiner characterizes as analogous to a generic ligand) and separating bound members of the library from unbound members to produce a sublibrary of polypeptides. The Examiner states that the selected sublibrary is then screened by contacting the library with a target and separating the library members bound to the target via their displayed polypeptides.

3. Prior to the April 4, 1997 filing date of the '098 patent, co-inventor Ian Tomlinson and I had conceived of the invention as claimed in claims 33-41 and 44-52. The invention was reduced to practice with diligence shortly thereafter. The attached Exhibit 1 consists of copies of Ian Tomlinson's notebook entries detailing the experiments, performed under our joint direction, that gave rise to the claimed invention. The dates on this exhibit have been redacted. A detailed description of these notebook entries and their relevance to the claimed invention is found in the accompanying Rule 131 Declaration of co-inventor Ian Tomlinson.

4. Co-inventor Ian Tomlinson and I had discussions on several occasions prior to April 4, 1997 regarding ways to improve polypeptide library technology. Specifically, we discussed the problems posed by the presence of a large background of library members that, due to the means used to introduce diversity (typically degenerate oligonucleotides and PCR), are not capable of proper folding and are therefore non-functional for binding to any target molecule. We recognized that a selection of library members for those capable of folding into a functional conformation would increase the likelihood of identifying library members that bind a desired target ligand.

Over the course of these ongoing discussions, the idea was developed that a functional conformation could be selected by selecting members of a library that bind a ligand generic to all functional members of the library. If proper folding is required for binding to the generic ligand, selection for such generic ligand binding would increase the proportion of molecules potentially in a conformation capable of binding a desired target. Selection performed on the resulting enriched library can identify functional members that also bind a desired target ligand. Thus, we had conceived of an approach for selecting a functional polypeptide library member from a repertoire comprising functional and non-functional members by selecting the repertoire with a generic ligand that only binds functional members, and then selecting the resulting pool of functional members for binding to a target ligand. One specific example of generic ligand binding we initially discussed was the binding of antibody molecules by superantigens, such as protein A and protein L. These proteins require that the antibody molecule be properly folded before they can bind. Therefore, such superantigen binding can be used to select library members that are properly folded.

We also recognized that the selection for folded, functional library members would have a particular impact on antibody libraries, because antibodies that have both Heavy and Light chains require the proper folding of both chains before they can bind a desired target ligand. Selection of a first selected pool of Heavy and/or Light chain polypeptides enriched for functional polypeptides, would be expected to increase the proportion of molecules potentially able to also bind a desired target ligand. One could then contact the first selected pool of polypeptides with a target ligand to select a population of polypeptides which bind to the target ligand. Thus, in our discussions prior to April 4, 1997, we conceived an approach to an improved antibody library in which a sub-library of antibody Heavy chains is selected for members that properly fold, a sub-library of Light chains is selected for members that properly fold, and the two selected sub-libraries are combined to form a library of antibodies that have been pre-selected for folded members that is then selected for binding to target antigen. The pre-selection for proper folding is performed by binding the members of each sub-library to a generic ligand that only binds properly folded sub-library molecules. For example, the Heavy chain sub-library can be pre-selected with Protein A, and the Light chain sub-library can be pre-selected with Protein L.

To bring our conception to fruition, Ian Tomlinson and I discussed, before April 4, 1997, the approach in which two scFv vector constructs are made, one encoding a "dummy" Heavy chain to be used to generate a sub-library of diverse Light chains, and one encoding a "dummy" Light chain to be used to generate a sub library of Heavy chains. Following selection for proper folding of the diverse domains by generic ligand binding of both sub-libraries, the "dummy" domain of one sub-library was to be replaced with the corresponding folding-selected domains from the other sub-library to generate a library of diverse, properly folded scFvs that could be selected for target binding.

Beginning before April 4, 1997, the experiments required to demonstrate this approach were undertaken under the direction of Ian Tomlinson. He and I conferred during the progress of the experiments until their successful completion after April 4, 1997, described in the notebook pages accompanying Dr. Tomlinson's Rule 131 declaration being filed concurrently with this declaration. I have reviewed the notebook pages and fully concur that our discussions and Dr.

Tomlinson's experimental work are in accord with both the notebook entries and Dr. Tomlinson's statements in his declaration.

5. I hereby declare that all statements made herein of my own knowledge are true, and that all statements made on information and belief are believed to be true; and further, that these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issuing thereon.

22/9/03
Date

G. P. Winter
Greg P. Winter